INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 13; Issue 1; January 2024; Page No.2755-2764
DOI: http://dx.doi.org/10.24327/ijcar.2024.2764.1601

Research Article

EVALUATE THE POTENTIAL OF CASSIA AURICULATA FLOWER EXTRACT AND APPLY TO CONTROLLING OF THE AIR BORNE PATHOGENS IN INDOOR SPACE USING A SPRAYING METHOD

Sangeetha.M[1], Muthu Lakshmi.P[2],Sindhuja.A[3] and Punitha.K[4]

Department of Microbiology, Kamaraj College, Thoothukudi -628003, Affiliated to MS University, Tirunelveli, Tamil Nadu.

ARTICLE INFO

Article History:
Received 23rd November, 2023
Received in revised form 8th January, 2024
Accepted 12th January, 2024
Published online 28th January, 2024

Key words:
Cassia auriculata, MIC and MBC, Antimicrobial activity, Air airborne pathogens and FT-IR and GC-MS.

ABSTRACT

People can become unwell from airborne microorganisms, and Cassia auriculata is used to treat many chronic ailments. Comparing the airborne microbe population before and after employing a spray was the study's main goal. Several extracts, including ethyl acetate, butyl alcohol, acetone, methanol, and distilled water, were tested for antimicrobial activity using the agar well diffusion method on bacteria like Escherichia coli, Staphylococcus sp., Pseudomonas sp., and Proteus sp. and fungi like Aspergillus sp. and Candida sp. The phytochemicals and functional groups were observed as GC-MS and FT-IR methanol flower extract revealed the functional group of the leaf extracts respectively. Thin layer chromatography and the Rf values of bioactive compounds were found. Escherichia coli (23 mm) and candida sp. (25mm) were detected in the floral extract of Cassia auriculata. We discovered MIC varies from 50µl to 400µl of 0.92 to 0.52 OD value and MBC ranges from 50µl to 400µl of 53 to NIL (CFU). In this test, the phytochemicals were found to exhibit antioxidant, anti-inflammatory, and anti-diabetic characteristics that were reflected in the methanol solvent. Using the settle plate method, the air contamination in a dangerous area was evaluated. As a result, we concluded that Cassia auriculata flower extract has the potential to be more successful in antimicrobial activity, controlling the air-borne pathogen and it contains various biological components.

INTRODUCTION

A germ that is spread through the air is the cause of an airborne disorder. Numerous pathogens, such as bacteria, viruses, and fungi, are aerosolized particles that cause many clinically significant airborne infections. (Fernstrom A, *et al.,* 2013). Major airborne transmitted pathogens in hospitals are Mycobacterium tuberculosis, Staphylococcus aureus, Influenza virus, Aspergillus flavus, A. fumigates, Candida albicans (Kausar *et al.,*2016) and Fungal genera e.g., Aspergillus sp., Penicillium sp., Alternaria sp., Talaromyces, Trichoderma, etc. are present predominantly in the haematological hospital environment (Cho *et al.,*2018). It's critical to understand that conditions brought on by toxins, smog, dust, or air pollution are not generally considered to be airborne diseases (Gammon J and Hunt J., 2018). The World Health Organization states that "Airborne transmission of infectious agents refers to the dissemination of droplet nuclei that remain infectious when suspended in air over long distances and time, resulting in the transmission of disease" (Seto WHO., 2015). Airborne pathogens are those that are produced in the respiratory system and spread through exhaled air (Morawska L *et al.,* 2005). Similar findings showed that a large portion of India's population lives in areas with poor air quality. The body becomes inflamed as a result of fine air pollution particles, notably the liver and lungs. According to a recent study, several plant extracts and oils include ingredients that help lessen the inflammation brought on by breathing in these polluting tiny particles (Kfoury M, *et al.,* 2016). When speaking, coughing, or even sneezing, the mouth is where the majority of aerial dispersal occurs, which largely uses saliva (Fiegel, *et al.,*). Human exposure to primary and secondary air pollutants can be increased by the chemical use of air fresheners. Exposure to air fresheners, even at low levels, has been linked to several negative health effects, including ventricular fibrillation, migraine headaches, asthma attacks, breathing problems, mucosal symptoms, dermatitis, infant diarrhoea and earaches, and dermatitis. Studies on the prevalence and different kinds of air freshener-related health consequences have recently been conducted (Steinemann., 2016). Natural room fresheners include Azadirachtaindica, Mentapiperita, and Aloe barbadensis plants. Although Cassia auriculata has a remarkable capacity to eradicate airborne pathogens, no air freshener has yet to be identified. Due to its higher cultural acceptance, better compatibility with the human body, and fewer side effects, herbal medicine continues to be the major form of primary healthcare for between 75 and 80 percent of the world's population, primarily in poor nations (Dr. Susan Sam., 2019). There is a high knowledge of the use and significance of these medicinal

Corresponding author:* **Sangeetha. M
Department of Microbiology, Kamaraj College, Thoothukudi -628003, Affiliated to MS University, Tirunelveli, Tamil Nadu

floras, despite the lack of scientific explanations for the therapeutic applications of plants by prehistoric people. This has prompted increased efforts to document therapeutic plants (Vandana Meena *et al.*,2019). In Ayurveda, the herb Cassia auriculata (CA) Linn, belonging to the Caesalpiniaceae family, is used to cure diabetes. It is also referred to as Tanner's Cassia, Tanner's Senna, Avaram tree or Avartaki in common parlance. Which can be found in the dry regions of southern, western, and central India and is grown in some areas of Punjab, Haryana, Uttar Pradesh, and West Bengal. Cassia fistula (Leguminosae) is frequently planted in gardens for adornment and as hedges. Flowers are enormous, a bright yellow shape, and irregular. Traditional herbal medicine uses parts of the Cassia auriculata plant, including the flowers, flower buds, roots, leaves, seeds, and bark, for a variety of purposes (Meena *et al.,* 2019). Over 40,000 distinct chemicals make up the huge family of secondary metabolites known as terpenoids (Aharoni. A. *et al.,* 2005). In various in vitro assays, monoterpenes, sesquiterpenes, and diterpenes have been demonstrated to have substantial anti-oxidant action. The majority of these activities, however, have no physiological significance. (Baratta, M.T *et al.,* 1998). One or more aromatic rings with one or more hydroxyl groups are typically seen in phenolic substances. The number of free hydroxyls and the conjugation of side chains to the aromatic ring have long been thought to boost the anti-oxidant potential of phenolics.According to reports, the plant contains antipyretic, hepatoprotective, antioxidative, and microbicidal properties. The flowers are used to cure throat infections and inflammation as well as nocturnal emissions, diabetes, urine discharges, and other conditions (Kumar RS, *et al.*,2002). The leaves treat ulcers, leprosy, and skin conditions and are anthelmintic. The flowers are utilized for diabetes, throat infections, and urine discharges.The fruit can help with sickness and thirst. Diabetes, diarrhoea, and chronic conjunctivitis can all be treated with the seed. It is thought that the bark is astringent (Subhadradevi *et al.*,2011).The airborne pathogens Bacillus subtilis,Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, and Aspergillusnigerare controlled by the aerial portions of Cassia auriculata. in the current study.Due to the development of resistance against widely marketed antibiotic formulations, an assessment of Cassia auriculata potential as an antibacterial agent is made using its flowers.Airborne pathogen needs to be controlled, which is crucial given the current circumstances. Future research will focus on anticancer activities, immunological investigation, testing our spray in outdoor settings, and commercial product release.

METHODOLOGY

Collection of samples

The completely developed Cassia auriculata flower was harvested in December from a nearby river. The plant's flower was collected, cleaned with sterile water two to three times to remove dust, and then shade-dried for 15 to 20 days at room temperature on sterile blotting paper. After complete drying, the plant material was ground into a powder using a blender and stored in a separate airtight container.

Solvent extraction

100ml of each of five different solvents, including ethyl acetate, butyl alcohol, acetone, methanol, and distilled water, were used to dissolve 10g of the dry powder of Cassia auriculata leaves. The extraction was carried out for 72 hours. The sample was extracted and then the Whatman No. 1 filtered paper was used to filter the sample. The filtrates were then placed in a refrigerator to be used later

Test microbes

The pure cultivation of bacteria such as Proteus spp., Staphylococcus spp., Pseudomonas spp., and Escherichia coli. pure cultures of fungus like Aspergillus species and Candida species. The organisms used for the antibacterial and antifungal assay were obtained from our microbiology laboratory.The test organisms were kept in the refrigerator on a nutrient agar slant.

Qualitative phytochemical analysis

The following phytochemical technique was used to analyses the obtained extracts for preliminary phytochemical screening.

Test for reducing sugar

Test tubes containing 1 ml of extract and 2-3 drops of Fehling's reagent should be mixed before being placed in a water bath for 1-2 minutes.Observe reddish-brown precipitate appearance denotes a successful outcome and the presence of reducing sugars.

Test for Flavanoids

A few drops of lead acetate solution were added to the extract for treatment.The presence of flavonoids is indicated by the observation of a precipitate with a yellow colour.

Test for saponins

In a test tube, 5 ml of diluted extract was added. The test tube was violently shaken for 5 minutes.Foamy layer formation indicates the presence of saponins.

Test for alkaloid

A few drops of Mayer's reagent were added to 1 mL of extract. A yellow precipitate that represents the presence of Alkaloids.

Test for quinines

One drop of concentrated sulfuric acid was added to 10 mg of each extract dissolved in isopropyl alcohol. The formation of red colour indicated the presence of quinones.

Test for protein

A few drops of the Biuret reagent were added to the tiny amount of extract. In the presence of protein, the blue reagent changed to violet.

Test for tannins

2 ml of 5% neutral ferric chloride solution was added to 1 mL of extract, the dark blue coloring indicating the presence of tannins.

Test for terpenoid

One ml of the extract from each solvent is combined with 0.5 ml of chloroform and a few drops of strong sulfuric acid. If a

reddish-brown precipitate forms, the extract contains terpenoids.

Anti-bacterial assay

The agar well diffusion method was used to test the antibacterial effects of extracts of ethyl acetate, butyl alcohol, acetone, methanol, and distilled water against Escherichia coli, Staphylococcus spp., Pseudomonas spp., and Proteus spp. Agar from Mueller Hinton was prepared, poured, and given time to set up. Escherichia coli, Staphylococcus sp., Pseudomonas sp. and Proteus sp., were fed into 24-hour cultures at various concentrations, such as 50µl, 100µl, 200µl, and 300 µl. For 24 hours, all of the plates were incubated at 37 degrees Celsius. After incubation, the zone of inhibition was assessed.

Antifungal assay

The agar well diffusion method was used to test the antifungal effects of extracts of ethyl acetate, butyl alcohol, acetone, methanol, and distilled water against Aspergillus spp. and Candida spp. Agar from Mueller Hinton was prepared, poured, and given time to set up. Aspergillus and Candida spp. cultures were placed onto plates for 24 hours at varying concentrations such as 50µl, 100µl, 200µl, and 300 µl and all the plates were incubated at 37 degrees Celsius.After incubation, the zone of inhibition was assessed.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

By using a broth dilution experiment, the minimal inhibitory concentration values were identified.Each 2mL of nutrient broth containing 0.1mL of standardized test organism of bacterial cells received varying quantities of the extract (50µl, 100µl, 200µl, 300µl, and 400µl) of each concentration. The tubes were incubated for 24 hours at 37°C.The smallest inhibitory concentration was determined by taking the tube with the least amount of extract that did not produce growth following incubation. Spread each concentration of the incubated tube on the nutrient agar. As the minimal bactericidal concentration (MBC), no growth plate was detected.

Anthelminthic activity

The anthelmintic activity of 0.1g/ml extracts of distilled water, butyl alcohol, and ethyl acetate from the Cassia auriculata flower was studied. As a positive control, albendazole at 0.01 g/ml was employed and distilled water as a negative control. Individual worms' paralysis and deaths were tracked in terms of timing. When no movement was observed consisted as paralysis unless the worms were violently shaking.When the worms didn't move when they were violently shaken, death had occurred.

Antioxidation determination assay

Phosphomolybdate assay (total antioxidant capacity)

The Phosphomolybdate assaywas used to measure the samples total antioxidant capacity. The reagent solution (.6 M (0.6 mole/L) sulfuric acid (H2SO4) 28mM (28 mmole/L) sodium phosphate (Na3PO4) 4 mM (4 mmole/L) ammonium molybdate.) was shaken with 0.1 ml of the sample solution. For 90 minutes, the test tubes were covered and heated to 95 °C in a water bath. The mixture's absorbance was gauged at 700 nm after the samples had cooled. Ascorbic acid served as the benchmark. The antioxidant capacity was estimated using the following formula:

Percentage inhibition= [(Absorbance of control − Absorbance of sample)/ (Absorbance of control)] × 100

Thin Layer Chromatography (TLC)

A glass sheet (slide) placed horizontally and covered with a thin coating of silica is known as a TLC plate. Using the capillary tube place a bit of sample in the bottom of the glass sheet. The TLC plate is then submerged in a beaker filled with a mixture of benzene/chloroform (1:1) and benzene/acetone (9:1). The mobile phase is liquid, also known as the eluent, which gently ascents the TLC plate through capillary action. The slide is withdrawn from the beaker, and the separated components of the mixture are viewed after the solvent has reached the top of the slide. Visualizing the compounds is simple if they are colored.

Rf = Distance traveled by the substance from reference line (cm) /Distance traveled by the solvent from reference line (cm)

Antidiabetic activity (Glucose-DNSA colour assay)

The chromogenic DNSA technique was used for the inhibition assay. One ml of salivary amylase, 500µl of 0.02 M sodium phosphate buffer (pH 6.9, 6 mmol sodium chloride), and 400µl of extracts with concentrations between 0.5 and 1.5 mg ml-1 (w/v) were added to a total assay mixture, which was incubated at 37 °C for 10 min. Each tube received 580µl of a 1% (w/v) starch solution after pre-incubation, and each tube underwent a 15-minute incubation at 37 °C. Each tube was then placed in a boiling water bath for 5 minutes to stop the reaction, cooled to room temperature, and the absorbance was measured at 540 nm. The reaction was then stopped by adding 1 ml of DNSA reagent. A control with no plant extracts demonstrated complete enzyme activity. Except for the enzyme, the reaction mixture also contained adequate extract controls to eliminate the absorbance caused by plant extract. Percent inhibition of alpha-amylase was calculated as follows:

%inhibition=(Abs(control)-Abs(extract) / Abs(control)) X100

Anti-inflammatory activity: (Egg albumin denaturation assay)

Egg albumin and phosphate-buffered saline (, pH 6.4) are combined in 0.2 ml. 2.8 ml and 2 ml of sodium diclofenac in various concentrations (10 g/ml, 20 g/ml, 30 g/ml, 40 g/ml, and 50 g/ml). As a control, prepare the same volume using double-distilled water. The mixes should be heated for five minutes at 70°C after 15 minutes at 37°C in the BOD incubator. At 660 nm, cool solutions and the measure absorbance. Calculate the percentage of inhibition of denatured protein by following the formula.

% inhibition = 100 × ([Vt/Vc] −1).

Where, Vt = absorbance of test sample,Vc = absorbance of control.

FTIR Spectroscopic Analysis

Perhaps one of the most effective techniques for determining the kinds of chemical bonds (functional groups) present in compounds is the Fourier transform infrared spectrophotometer (FTIR). For the FTIR study, dried powders

of various solvent extracts of each plant material were employed. To create a translucent sample disc, 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet plant specimen's powdered sample was placed into an FTIR Spectroscope with a 400 to 4000 cm-1 scan range and a 4 cm-1 resolution.

Gas Chromatography-Mass Spectrometry Analysis

The sample was diluted with methanol to a volume of 1 mL from 20 microliters. The column was an RTX-5MS column with dimensions of 30 m, 0.25 mm i.d., and 0.25µL film thickness. Helium carrier gas (99.99%, AGA Lithuania) flow rate was set to 1.23 mL/min. Following injection, the oven temperature was held at 40 °C for 2 minutes before being programmed to rise by 3 °C per minute to 210 °C, where the column was kept for 10 minutes. The split was 1:10 in ratio. The electron ionization of the mass detector was 70 eV. Mass spectra library search (NIST 14) was used to identify volatile chemicals, and the results were compared to mass spectral data from the literature.

Controlling Airborne Pathogens Using Spray

Preparation of the spray

100ml of water should be added to 10g of dry Cassia auriculata powder. The mixture was heated and passed through a filter of filtered paper. 10 drops of fragrance oil (javathu) should be added to the filtered solution and used for this experiment by pouring it into the spray bottle

Microbial contamination test in different locations

The investigation was carried out in our college's indoor settings. Phase 1 was carried out in a lab setting under controlled conditions to examine how spray affected airborne microorganisms. Phase 2 was carried out in our classroom. Phase 3 was carried out in the vaccination chamber. Phase 4 was carried out in the classroom. Phase 5 was carried out in the classroom. Place the nutrient plate outside for five minutes to prepare for spraying. At the designated location, the nutrient agar was exposed, and the lid was slowly removed without contacting the media. Place the Petri plate for 15 minutes. After that, slowly shut the lid and let it sit for 24 hours. Spray the solution on every side and corner of the room. After the spray, wait for 15 minutes and repeat the process in other fresh nutrient agar plates. Incubate the plate for 72 hours at room temperature. Observe the colony and count the number of microbial colonies. Calculate the percentage of inhibition of microbial load by following the formula.

Percentage of reducing microbial load = Before spray – after spray)/ Before spray x100

RESULT AND DISCUSSION

Quality of phytochemical analysis

In the ethyl acetate, tannins, flavonoids, and reducing sugar were present, while terpenoids were not. Tannins, flavonoids, reducing sugar, terpenoids, saponins, and alkaloids are all present in the ethanolic extract, whereas protein is absent. Tannins and flavonoids were present in the methanol, but terpenoids and reducing sugar were not. (Guruprasad C.Nile and K.R.C.Reddy.,2015). The present study shows the presence of reducing sugar, alkaloids, flavonoids, tannins, terpenoids and quinones and the complete absence of protein and saponin was observed in the different extract was shown in table 1. The study investigated the presence or absence of this component in the plant flower material of Cassia auriculata.

Table 1 Phytolchemical analysis for different extracts of cassia auriculata

Phytochemical	Ethyl acetate	Butyl alcohol	Acetone extract of	Methanol extract of	Distilled water
Reducing sugar	+	+	+	+	+
Tannis	+	+	-	+	-
Flavonoid	+	+	-	+	-
Terpenoid	+	+	+	+	+
Alkaloid	+	+	+	+	+
Saponin	-	-	-	-	-
quinones	+	+	+	+	+
Protein	-	-	-	-	-

Antibacterial activity

Antimicrobial activity of the methanolic extract of Cassia auriculata flowers was found to have higher inhibitory activities against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi. (Gaurav M. Doshi *et al.*, 2011). The present study shows that the extraction of Cassia auriculata was evaluated in different concentration for antibacterial activity Escherichia coli, Staphylococcus sp, Pseudomonas sp and proteus sp.Indicating the different zone of inhibition was shown in table 2 and figure 1-4. Our result revealed that ethyl acetate shows signification higher inhibitory activities against Escherichia coli (23mm).

MIC and MBC activity

The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and extract were reported (Gaurav M. Doshi *et al.*, 2011). In the present study ethyl acetate extract from flower was found higher inhibitory and bactericidal activities against Escherichia coli and Staphylococcus sp.The minimum inhibitory concentration at 300 µl and minimum bactericidal concentration at 400 µl was shown in table 3 and figure 5.

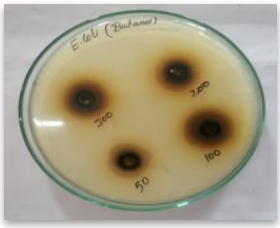
Table 2 Antimicrobial activity of different extract of cassia auriculata flower

Bacterial Strains	Concentration of plant extraction	Zone of inhibition of different (mm)				
		Ethyl acetate	Butyl alcohol	acetone	methanol	Distilled water
Escherichia coli	50 µl	19	14	12	12	16
	100 µl	20	19	14	13	17
	200 µl	21	21	15	14	19
	300 µl	23	22	16	17	20
Staphylococcus sp.	50 µl	18	17	13	13	14
	100 µl	20	18	15	16	16
	200 µl	21	19	16	17	18
	300 µl	22	22	18	18	19
Pseudomonas sp.	50 µl	16	11	13	12	15
	100 µl	17	12	16	13	17
	200 µl	18	14	17	14	19
	300 µl	19	15	18	16	20
Proteus sp.	50 µl	15	13	11	12	10
	100 µl	17	14	12	16	12
	200 µl	18	15	13	17	13
	300 µl	20	17	15	19	14

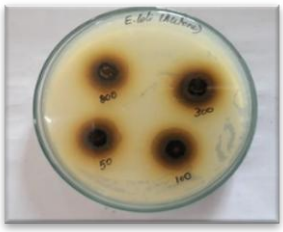
Antibacterial Activity

Figure 1 *Escherichia coli*

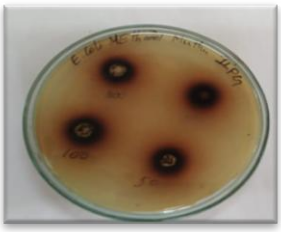
(a)Ethyl acetate



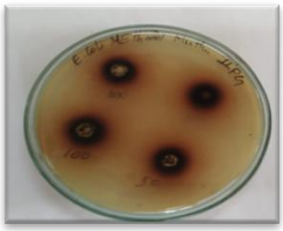
(b)butyl alcohol



(c) acetone

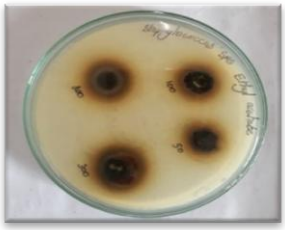


(d) methanol

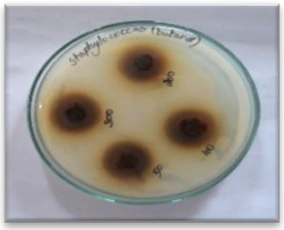


(e) distilled water

Figure 2 *Staphylococcus sp*

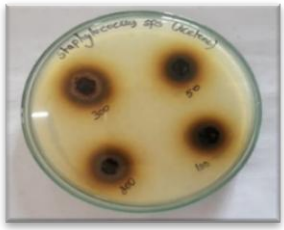


(a)Ethyl acetate

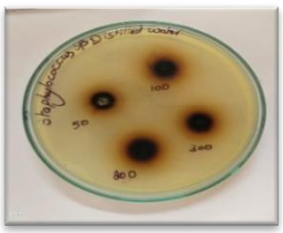


(b)butyl alcohol

(c) acetone



(d) methanol



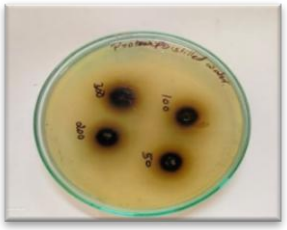
(e) distilled water

Figure 3 *Pseudomonas sp.*

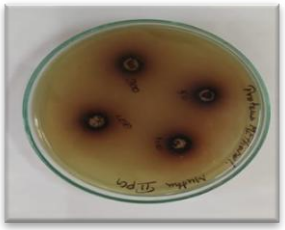
(a)Ethyl acetate

(b)butyl alcohol

(c) acetone



(d) methanol



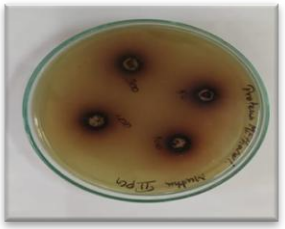
(e) distilled water

Figure 4 *Proteus sp*

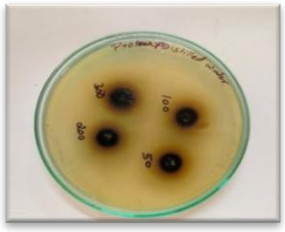
(a)Ethyl acetate

(b)butyl alcohol

(c) acetone



(d) methanol

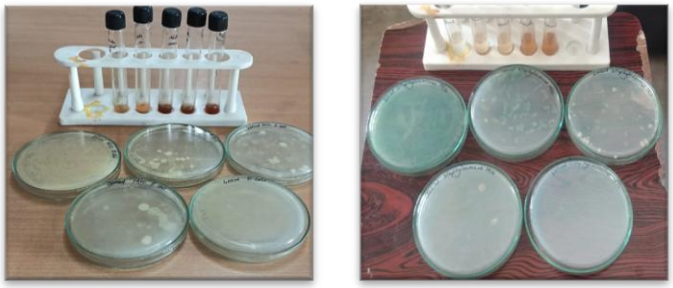


(e) distilled water

Table: 3 MIC and MBC of ethyl acetate extract of different concentrations of cassia auriculata

Bacterial strains	Concentration of ethyl acetate extract	MIC OD value	MBC (CFU)
Escherichia coli	50 µl	0.92	67
	100 µl	0.81	43
	200 µl	0.72	19
	300 µl	0.61	4
	400 µl	0.52	Nil
Staphylococcus sp.	50 µl	0.95	53
	100 µl	0.86	33
	200 µl	0.66	21
	300 µl	0.51	3
	400 µl	0.41	Nil

Figure 5 MIC and MBC of ethyl acetate extract of different concentration of *cassia auriculata*

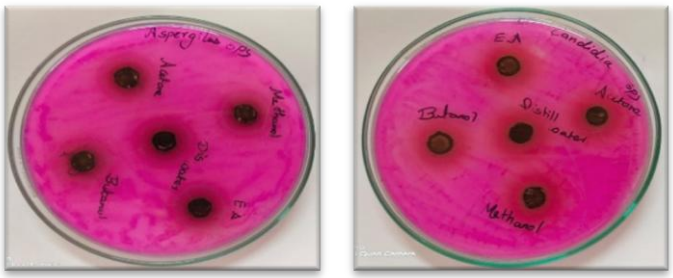


(a) Escherichia coli (b) Staphylococcus sp.

Antifungal activity

Standard fungal cultures Aspergillusniger and Trichodermaviride were used. Antifungal screening is performed by the above explained well diffusion method (Jesteena *et al*., 2016). The presence shows the ethyl acetate, butyl alcohol, acetone, methanol and distilled water extraction of Cassia auriculata evaluated antifungal activities against Aspergillus sp. and candida sp. **table 4 and figure 6**

Figure 6 Antifungal activity



(a) Aspergillus sp. (b) candida sp.

Table 4: Antifungal activity

Strains	Zone of inhibition of different (mm)				
	Ethyl Acetate	Butyl Alcohol	Acetone	Methanol	Distilled Water
Aspergillus sp.	24	24	23	21	20
candida sp.	25	24	23	20	19

Anthelmintic activity

Ethyl acetate extract exhibits better anthelmintic activity than the standard. In the case of petroleum ether extract, paralysis was caused earlier but death time was longer. In the case of ethanol extract, the paralysis time was longer at lower doses [20 mg/ml] but shorter at higher doses [40-60 mg/ml] (SushmaKainsa *et al.,* 2012). The present study showed that the Ethyl Acetate, butyl alcohol, acetone and distilled water of Cassia auriculata flowers exhibit anthelminthic activities extraction dependent manner. The Albendozole solution is a positive control. The distilled water is a negative control. All are shown in the **table 5.**

Table 5 MBC of ethyl acetate extract of different concentrations of cassia auriculata

Different extraction	Paralysis (min)	Death (mm)
Ethyl acetate	10	12
Butyl alcohol	12	14
Acetone	10	12
Distilled water	13	15
Albendazole (positive control)	186	198
Distilled water (negative control)	0	0

Thin layer chromatography

Methanol extract of *Cassia auriculata* gives three different type of compounds which is shown. Rf value for the corresponding solute and solvent can be calculated. Hence it confirms the presence of flavonoids and terpenoids. (M. Monisha *et al.,).*The present study on ethyl acetate, butyl alcohol and acetone extract of *Cassia auriculata* gives different compound which is shown RF value for the corresponding solute and solvent can be calculate and the value are shown in table 6. The previous study confirmed that the present of flavonoids and terpenoids and present study show the same result.

Table 6 Determination of bioactive compounds through TLC

Solvent	sample	No of spot	RF value
Benzene: chloroform	Ethyl acetate	2 spots	0.38
			0.43
	Butyl alcohol	1 spot	0.38
	acetone	2 spots	0.46
			0.58
Benzene: acetone	Ethyl acetate	2 spots	0.33
			0.63
	Butyl alcohol	1 spot	0.55
	acetone	2 spots	0.36
			0.65

Antioxidant activity

This antioxidant activity is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically shows antioxidant capacity of fractions of M. buxifolia fruit in the order of aqueous > methanol > butanol > ethyl acetate > n-hexane

fractions. The aqueous fraction with an EC50 value of 45.2 2.53) showed high antioxidant capacity followed by the methanol fraction (56.4 2.06). (Shumaila Jan *et al.*, 2013). The methanolic fraction shown the antioxidant capacity. The positive control is ascorbic acid. The percentage inhibition of phosphomolybdate assay in methanol showed 45.88% in a minimum concentration (100 µl) and 72.36%in a (maximum concentration 500 µl shown in table

Table 7 Antioxidant activity of methanol extract of cassia auriculata flower

Concentration of the sample	Standard OD Value(ascorbic acid)	Sample OD value	% of inhibition
100µl	0.732	0.442	45.88%
200µl	0.702	0.386	52.49%
300µl	0.632	0.316	59.36%
400µl	0.505	0.256	61.48%
500µl	0.356	0.226	72.36%

Anti-inflammatory activity

Ethanolic crude extract of Cassia auriculata flowers exhibited significant anti-inflammatory activity result showed the Percentage of Inhibition in membrane Stabilization for 100 µg/ml as 29.15±1.75%, for 200 µg/ml as 49.18±1.87%, for 400 µg/ml as 58.26±1.58%, for 600 µg/ml as 85.16±0.49 and for 800 µg/ml as 89.24±1.70%. (Murugananthamet al.;2015). The result showed the percentage of inhibition in membrane stabilization for 200 µl as 42.52%, for 400 µl as 50.53%, for 600 µl as 54.44%, for 800 µl as 60.14% and 1000 µl as 65.30%. The present study shows of the minimum inhibition percentage 42.52% in 200 µl and the maximum inhibitory percentage 65.30% in 1000 µl shown in **table 8.**

Table 8 Anti-inflammatory activity of methanol extract of cassia auriculata flower

Concentration of the sample	OD Value for sample	Inhibition percentage
200 µl	0.423	42.52%
400 µl	0.378	50.53%
600 µl	0.356	54.44%
800 µl	0.324	60.14%
1000 µl	0.295	65.30%

Antidiabetic activity

Based on the higher alfa amylase activities of Ethanolic Extract of Cassia auriculata have been used for In Vivo studies. The inhibition percentage of amylase activity and α-glucosidase activity is 33.6%75(µg/ml),55%in 100(µg/ml) and 62% in125(µg/ml). (G. Nagaraja Perumal *et al.*, 2021).In the present study, were investigated for their potential to inhibit α amylase activity. Five different concentrations viz., 200 µl, 400 µl, 600 µl,800 µl and 1000 µl of test samples were separately tested for the inhibition of α-amylase activity and α-glucosidase activity along with control. The inhibition percentage of α-amylase activity and α-glucosidase activity is 23.70% in 200 µl, 28.26% in 400 µl,39.70% in 600 µl,43.46% in 800 µl and 49.54% in 1000 µl. The inhibition percentage of α-amylase activity of lower concentration level 23.70% in 200 µl and higher concentration level 49.54% in 1000 µl was shown in **table 9.**

Table 9: Anti-diabetic activity of methanol extract of cassia auriculata flower

Concentration of the sample	OD value of sample	Percentage inhibits of amylase
200 µl	0.523	23.70%
400 µl	0.472	28.26%
600 µl	0.400	39.70%
800 µl	0.372	43.46%
1000 µl	0.332	49.54%

FTIR of methanol extract of cassia auriculata

As per FTIR spectra of Cassia auriculata test sample extract, the functional groups of the components were separated based on their peak ratios. The result confirmed the presence of characteristic band at 3007.55 cm-1, 2922.45 cm1 ,2853 cm-1 shows the C-H stretching, at 2378.10 cm-1 , 2310.22 shows -P-H stretching, at 1742cm -1 shows the C=O stretch carboxylic acid / Ketone group, at 3397.87cm-1 shows O-H stretch group, at 1457.46 cm-1 shows C-H bending, at 1055.21 cm-1, 1032.84cm-1, 1097cm-1 shows C-C stretch group.(Jesteena *et al.*, 2019). The present study show the different functional group of the component were separated based on their peak ratios. The result shows that the present of characteristic band at. the present study shows that the extra presents of components such as Alkyl halide, C-Br stretching, Alkene, Anhydride, Sulfoxide, Secondary alcohol, Alkyl amine, Alkyl ketone, Phenol, C-O,Sulfonamide, Alkanes, N-O stretchingnitro compound, α,β-unsaturated ketone, C=O stretching (cyclopentanone), O=C=O stretching (carbon dioxide), C-H stretching alkane, N-H stretching aliphatic primary amine and O-H stretching.

TABLE 10 FTIR peak value of methanolic extract of cassia auriculata

S.NO	PEAK VALUE	FUNCTIONAL GROUP
1	456.13	Alkyl halide
2	517.85	Alkyl halide
3	592.11	C-Br stretching
4	836.08	Alkene
5	959.92	Alkene
6	1011.59	Anhydride
7	1071.38	Sulfoxide
8	1115.74	Secondary alcohol
9	1158.17	Alkyl amine
10	1232.43	Alkyl ketone
11	1317.29	Phenol
12	1338.51	C-O
13	1363.58	Sulfonamide
14	1455.19	Alkanes
15	1511.12	N-O stretching nitro compound
16	1622.02	α, β-unsaturated ketone
17	1745.46	C=O stretching (cyclopentanone)
18	1790.78	C=O stretching conjugated acid halide
19	2360.71	O=C=O stretching

		(carbon dioxide)
20	2919.06	C-H stretching alkane
21	3444.63	N-H stretching aliphatic primary amine
22	3737.79	O-H stretching

GCMS of methanol extraction of cassia auriculata flower.

The potential antibiotic and radical scavenge of F3 might be the action of Di-(2-ethylhexyl) phthalate and 1,2-Benzenedicarboxylic acid were confirmed by GCMS (S. Senthilrani and P.Renuka Devi 2014).

Table 11 GC-MS analysis of methanol extraction of *cassia auriculata* flower

Name of the compound	**R T**	**Area**	**Molecular formula**	**Percentage of area**	**Quality**
Pentanoic acid, 1-methylethyl ester	9.207	706828	$C_8H_{16}O$	19.58	*27*
Butanoic acid, 3-methyl-, 1-meth	9.207	706828	$C_5H_{10}O_2$	19.58	*27*
2-O-Methyl-D-mannopyranosa	9.207	706828	$C_7H_{14}O_6$	19.58	25
5-Nonenal, (E)-	14.105	121105	$C_9H_{16}O$	3.35	36
3-Tetradecyn-1-ol	14.105	121105	$C_{14}H_{26}O$	3.35	25
1,2-Cyclohexanediol, cyclic sulf	14.105	121105	$C_6H_{10}O_3S$	3.35	25
Bicyclo(3.3.1)nonane-2,6-dione	15.58	34330	$C_9H_{12}O_2$	0.95	9
Bicyclo[3.3.1]nonane-2,7-dione	15.58	34330	$C_9H_{12}O_2$	0.95	9
Bicyclo(3.3.1)nonane-2,6-dione	15.58	34330	$C_9H_{12}O_2$	0.95	9
1,5,7-Octatrien-3-ol, 2,6-dimethylethyl	15.617	46457	$C_{10}H_{16}O$	1.29	*12*
2-Methyl-3-nitroaniline	15.617	46457	$C_7H_8N_2O_2$	1.29	9
Cyclohexanone, 5-methyl-2-(1-methylethyl	15.617	46457	$C_{10}H_{18}O$	1.29	*9*
3-Ethoxy-1,4,4a,5,6,7,8,8a-octah	15.665	188156	$C_4H_5NO_2S_2$	5.21	35
Oxirane, 3-[5-(4-azidophenoxy)-3...	15.665	188156	$C_{13}H_{15}N_5O_4$	5.21	10
2-Dodecen-1-yl(-)succinic anhydride	15.665	188156	$C_{16}H_{26}O_3$	5.21	10
Benzene, 1-thiobenzoyl-2,4,6-tri...	17.783	212543	$C_{15}H_{24}S$	5.89	59
o-(p-(Dimethylamino) benzylidenea...	17.783	212543	$C_{15}H_{16}N_2O$	5.89	53
Chalcone, 2',4'-dihydroxy-	17.783	212543	$C_{15}H_{12}O_3$	5.89	53
1,1,1,5,7,7,7-Heptamethyl-3,3-bi...	18.917	1121276	$C_{12}H_{24}B_2O_4$	31.06	58
Heptasiloxane, hexadecamethyl-	18.917	1121276	$C_{16}H_{48}O_6Si_7$	31.06	45
3,6-Dioxa-2,4,5,7-tetrasilaoctan...	18.917	1121276	$C_{12}H_{30}O_2Si_2$	31.06	22
Cyclononasiloxane, octadecamethyl-	18.955	198785	$C_{18}H_{54}O_9Si_9$	5.51	50
Cyclononasiloxane, octadecamethyl-	18.955	198785	$C_{18}H_{54}O_9Si_9$	5.51	50
Hexasiloxane, tetradecamethyl-	**18.955**	**198785**	$\mathbf{C_{14}H_{42}O_5Si_6}$	**5.51**	**50**
Cyclononasiloxane, octadecamethyl-	18.984	373504	$C_{18}H_{54}O_9Si_9$	10.35	53
Hexasiloxane, tetradecamethyl-	18.984	373504	$C_{14}H_{42}O_5Si_6$	10.35	37
Cyclononasiloxane, octadecamethyl-	18.984	373504	$C_{18}H_{54}O_9Si_9$	10.35	37
Pentasiloxane, dodecamethyl-	19.040	607088	$C_{12}H_{36}O_4Si$	16.82	27
Pentasiloxane, dodecamethyl-	19.040	607088	$C_{12}H_{36}O_4Si$	16.82	27
Silane, [[4-[1,2-bis[(trimethyls...	19.040	607088	$C_{15}H_{25}NO_2SSi_2$	16.82	25

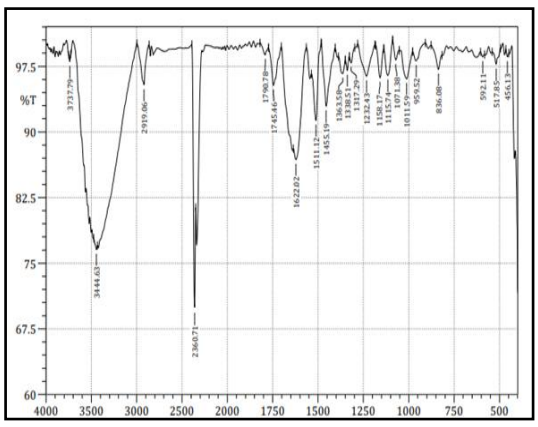


Figure 7 FTIR of methanol extraction of cassia auriculata flower

The present study shows that the present of different components was shown in the table 11 and figure 8

Controlling of air borne microbes

The air microbial monitoring was done within a controlled environment. Viable microbes were reported before spray and 4 h after spray shin 3.6×2.4 m room.There was a reduction of 68-93% microbes after spray in the controlled laboratory room. (Amul S Bahl 2019). The present study contacted in five phases-controlled environments. Viable microbe was reported before spray and after spray in a specific room. The inhibition of air borne pathogen in room 1 is 71%, room 2 is 60%, room 3 is 73%, room 4 is 80% and room 5 is 78%. The test shows a significant change towards the low level of microbe with the application of the indoor environment.

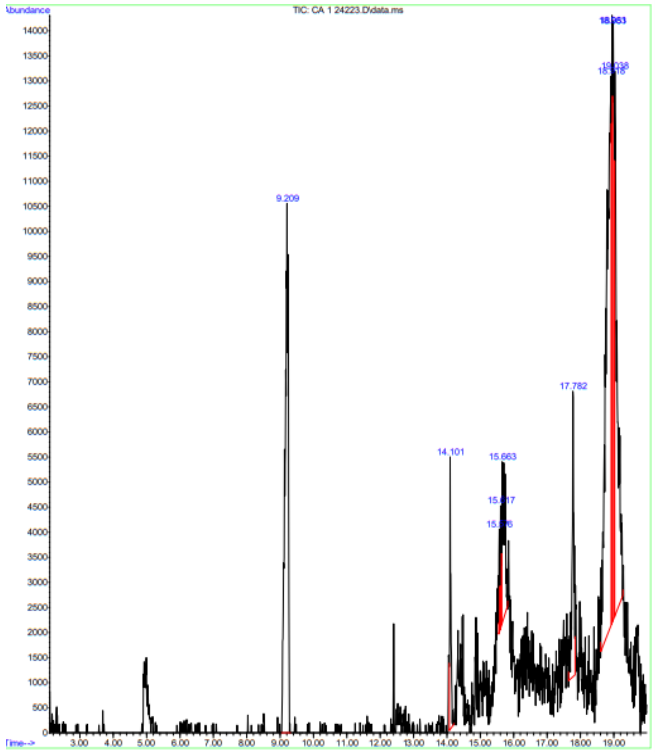


Figure 8 GCMS of methanol extraction of cassia auriculata flower

Table 12 Evaluation of microbial load of before and after spray

Different location	Before spray of microbial count	After spray of microbial count	Percentage of inhibition of air borne microbes
Room1	58	18	71%
Room2	45	19	60%
Room3	56	15	73%
Room4	64	13	80%
Room5	65	14	78%

CONCLUSION

The flower of the Cassia auriculata displayed biological activity, including the presence of phytochemicals. It displayed the highest zone of bacterial and fungal inhibition. The current study demonstrated the Cassia auriculata flower's antioxidant, anti-inflammatory, and anti-diabetic properties, as well as its anthelminthic properties. Thin layer chromatography, FTIR, and GCMS were used to identify the chemical structure, bioactive compound names, and function group. A spray made from the Cassia auriculata flower further shows its effectiveness in decreasing airborne pathogens. Future research will focus on the biological mechanisms underlying anti-tumor, anti-cancer, anti-toxic, and anti-toxicity effects, which are crucial for large-scale industries, medical use, and environmental applications.

References

1. Fernstrom A and Goldblatt M. (2013). Aerobiology and its role in the transmission of infectious diseases. J Pathog. 2013:493960
2. Kausar MA, Arif JM, Alanazi SMM, Alshmmry AMA, Alzapni YAA, Alanazy FKB, Shahid SMA and Hossain A (2016) Assessment of microbial load in indoor environment of university and hospitals of hail, KSA Biochem cell Arch Vol. 16, No. 1, pp. 177-183, 2016
3. Cho SY, Myong JP, Kim WB et al (2018) Profiles of Environments Mold; Indoor And Outdoor Air Sampling In A Haematology Hospital In Seoul, South Korea,Int J Environ Res Public Health 15;2560.
4. Gammon J and Hunt J., (2018) A review of isolation practices and procedures in healthcare settings. Br J Nurs. ;27(3):137-14
5. Seto WH. (2015) Airborne transmission and precautions: facts and myths. 2015 J Hosp Infect. Apr;89(4):225-8
6. Morawska L. (2005) Droplet fate in indoor environments, or can we prevent the spread of Infection? Proceedings Indoor Air. 2005:9–23.
7. Kfoury, M. et al. (2016). Essential oil components decrease pulmonary and hepatic cells inflammation induced by air pollution particulate matter, Environmental Chemistry Letters. DOI 10.1007/s10311-016-0572-4
8. Fiegel J, Clarke R, Edwards DA. (2006) Airborneinfectiou disease and the suppression of pulmonary bioaerosols. Elsevier; vol. 11(1/2). p. 51–7.
9. Steinemann , (2016) Fragranced consumer products: exposures and effects from emissions Air Quality, Atmosphere & Health volume 9, pages861–866
10. Dr. Susan Sam., (2019) Importance and effectiveness of herbal medicines Journal of Pharmacognosy and Phytochemistry Vol. 8, Issue 2
11. VandanaMeena, HimangshuBaruah and Rehana Parveen (2019)Cassia auriculata: A healing herb for all remedy Journal of Pharmacognosy and Phytochemistry; 8(3): 4093-4097
12. Meena V, Baruah H, and Parveen R (2019) Cassia auriculata: A healing herb for all remedy. J PharmacognPhytochem 8, 4093–4097.
13. Aharoni, A., M.A. Jongsma and H.J. Bouwmeester, (2005) Volatile science? Metabolic engineering of terpenoids in plants. 2005. Trends Plant Sci., 10: 594-602.
14. Baratta, M.T., H.J.D. Dorman, S.G. Deans, D.M. Biondi and G. Ruberto, (1998) Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. J. Essent. Oil 1998. Res., 10: 618-627.
15. Kumar RS, Ponmozhi M, Viswanathan P, Nalini N. (2002) Effect of Cassia auriculata leaf extract on lipids in rats with alcoholic liver injury. Asia Pac J ClinNutr ; 11:157 63.
16. Subhadradevi, V., Khairunissa, K., Asokkumar, K., Umamaheswari, M., Sivashanmugam, A., Jagannath, P (2011) Induction of apoptosis and cytotoxic activities of Apium graveolens Linn. Middle-East J. Sci. Res. 9 (1), 90-94.
17. GuruprasadC.Nile and K.R.C.Reddy., (2015) Guruprasad C. Nille1, K.R.C. Reddy 2 A Phytopharmacological Review of Plant – Cassia auriculata, International Journal of Pharmaceutical &

Biological Archives; Available Online at www.ijpba.info volume 6 (6): 1 –9
18. Jesteena, Johney, Kannan, Eagappan,Ragunathan, R.R (2017) Microbial extraction of chitin and chistosan from Pleurotus spp.,its characterization and antimicrobial activity. 2017 Int J Curr Pharm. Volume 9(1) page 88-93.
19. Gaurav M. Doshi, Supriya S. Shidhaye, Gayatri V. Aggarwal, Preeja P. Pillai1, Abhijeet B. Bhalerao, Sandhya K. Desai (2011) Antibacterial potential of Cassia auriculataflowers. Journal of Microbiology and Biotechnology Research Scholars ResearchLibrary J. Microbial. Biotech. Res.ISSN: 2231 –3168 CODEN (USA): JMBRB4 volume 15. 1 (3): 15-19
20. Jesteena, Johney, Kannan, Eagappan,Ragunathan, R.R (2016) Microbial extraction of chitin and chistosan from Pleurotus spp., its characterization and antimicrobial activity. 2017 In J Curr Pharm. Volume 9(1) pages 88-93.
21. SushmaKainsa, Praveen Kumar and Randhir Singh Dahiya (2012) Investigation of in vitro anthelmintic activity of Cassia auriculata leaves http://scholarsresearchlibrary.com/archive. Html ISSN: 2231 – 3184 CODEN (USA): JNPPB7 460 Scholars Research Library, 2 (4):460-464
22. M. Monisha, M. Sowmiya1, R. Ragunathan and JesteenaJohney (2017) Extraction of Bio-Active Compounds from Cassia auriculata Pods and Leaves and its Medicinal Uses International Journal of Current Microbiology and Applied Sciences ISSN: 2319- 7706 Volume 6 Number 8 pp. 425-434.
23. Shumaila Jan, Muhammad Rashid Khan Umbreen Rashid, JasiaBokhari (2013) Assessment of Antioxidant Potential, Total Phenolics and Flavonoids of Different Solvent Fractions of MonothecaBuxifolia Fruit 2013 http://dx.doi.org/10.1016/j.phrp.2013.09.003, pISSN 2210-9099 eISSN 2233-6052
24. Muruganantham *et al.*, 2015 Muruganantham S, Anbalagan G, Ramamurthy N. FT-IR and SEM-EDS comparativeanalysis of medicinal plants, Eclipta alba Hassk and Ecliptaprostrata Linn. 2009 Rom J Biophys; 19:285-94.
25. Jesteena, Johney, Kannan, Eagappan,Ragunathan, (2019) R.R Microbial extraction of chitin andchistosan from Pleurotus spp., its characterization and antimicrobial activity. 2017 Int J Curr Pharm. Volume 9(1) pages 88-93.
26. Nagarajaperumal G, Idachristi V. E, Karpakavalli M, Mohan S. In Vitro and In Vivo Evaluation of Potential Anti Diabetic Efficacy on Cassia Auriculata Flowers. 2018Biomed Pharmacol J;11(4).
27. S.Senthilrani and P. Renuka Devi(2014). Biological activities and chemical composition of cassia auriculata, Asian Journal of Biochemistry 9 (4): 195-202,2014.
28. Amul S. Bahl., (2019) Impact of plant-based natural extracts on pollutants and pathogens in the air 2019 Journal of Air Pollution and Health Original Article Available online at volume 4(3): 15

How to cite this article:
Sangeetha. M,Muthu Lakshmi. P, Sindhuja. A and Punitha.K., 2023, Evaluate the Potential of Cassia Auriculata Flower Extract and Apply to Controlling of the Air Borne Pathogens in Indoor Space Using a Spraying Method. *International Journal of Current Advanced Research.*13 (1), pp.2755-2764.

****